In vitro antifungal activity of soil fungi crude extracts isolated from riparian forest against plant pathogenic fungi

Jantasorn, A., Mongon, J., Moungsrimuangdee, B. and Oiuphisittraiwat, T.

ABSTRACT

Fungal diseases affecting plants are one of the most destructive diseases and cause significant losses in many economic crops in Thailand and worldwide. The most common plant pathogenic fungi which cause severe diseases to economic crops in Thailand are *Lasiodiplodia theobromae, Sclerotium rolfsii, Phytophthora palmivora, Colletotrichum capsici, Pyricularia grisea, Alternaria* sp., *Helminthosporium maydis, Rhizoctonia solani, Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Control of these pathogens is carries out through the use of synthetic fungicides which are harmful to environment. This study aims to at investigating the efficacy of the ethyl acetate crude extracts of the cultures *Talaromyces flavus* Bodhi003, *Neosartoryafischeri*Bodhi004 and *Eurotium* sp. Bodhi005 isolated from riparian forest soils to inhibit the mycelial growth of ten plant pathogenic fungi in *in vitro* conditions at various concentrations. At the highest concentrations (10,000 ppm), all crude extracts exhibited a complete mycelial growth inhibition of some plant pathogenic fungi when compared with the water control. Interestingly, *Eurotium* sp. Bodhi005 crude extract was recorded as having excellent inhibitory activity against *S. rolfsii* at 1,000 ppm concentration. Results from this study demonstrate that ethyl acetate crude extracts from *T. flavus* Bodhi003,*N. fischeri*Bodhi004, and *Eurotium* sp. Bodhi005 could be used to control the mycelial growth of plant pathogenic fungi and allow researcher to identify new potential sources for the development of alternative bio-fungicide to reduce plant pathogenic fungi.

MS History: 15.06.2016 (Received)-30.08.2016 (Revised)- 20.10.2016 (Accepted)

Key words: Soil fungi, *Talaromyces flavus* Bodhi003, *Neosartorya fischeri* Bodhi004, *Eurotium* sp. Bodhi005, plant pathogenic fungi.

Citation: Jantasorn, A., Mongon, J., Moungsrimuangdee, B. and Oiuphisittraiwat, T.. 2016. *In vitro* antifungal activity of soil fungi crude extracts isolated from riparian forest against plant pathogenic fungi. *Journal of Biopesticides*, 9 (2): 119-124.

INTRODUCTION

Plant diseases caused by fungi are one of the most destructive pathogens and cause significant losses in many economic crops in Thailand and worldwide. The fungi generate the greatest impact, in terms of a reduction in crop productivity, estimated to be about 14% and lead to huge losses to mankind (Agrios, 2005; Tapwal *et al.*, 2011). The important plant pathogenic fungi which cause severe diseases to economic crops are as follows: *Phytophthora palmivora* Butler. causes root and stem rot, *Colletrichum* species are responsible for anthracnose disease, and *Pyricularia grisea* Sacc causes rice blast. In addition, *Sclerotium rolfsii* Sacc. and *Fusarium oxysporum* (E.F. Sm. & Swingle) are reported to be the most destructive pathogens and cause the greatest impact resulting in yield losses (Couch *et al.*, 2002; Al-Askar, 2012; Alwathnani and Perveen, 2012; Abdel-Fattah *et al.*, 2011).

Management of these pathogens is required, the antifungal activity from the fruit extracts of *Hydnocarpus anthelminthicus* Pierre ex Laness and *Xanthophyllum lanceatum* J. J. Sm. collected from riparian forest have been reported to be effective against *P. oryzae*, *P. palmivora, R. solani* and *S. rolfsii* (Jantasorn *et al*., 2016).The applications of soil fungi crude extracts are carried out for the prevention and control of plant diseases as new potential sources for the development of alternative bio-fungicide replace synthetic fungicide. The antifungal activity from *N. pseudofischeri* KUFA0060 and *N. quadricincta* KUFA0064 have been reported to be effective against plant pathogenic fungi (Boonsang *et al.*,

2014). However, the soil fungi extracts have been identified as sources of bio-pesticide for the purpose of decreasing the dependency on chemical pesticide, developing a new environmentally safe bio-pesticide that is easily degradable, non-toxic, and less hazardous to human health. Nevertheless, there are only a few reports regarding antifungal activity of soil fungi against plant pathogenic fungi that have been collected in Thailand and there are merely some genera of soil fungi namely, *Neosartorya*, *Eupenicillium* and *Gelasinospora* that have been reported to produce bioactive compounds with biological activities (Kaewchai *et al.*, 2009; Talubnak and Soytong, 2010; Sibounnavong *et al.*, 2012; Eamvijarn *et al*, 2012, 2013). Besides the antimicrobial activity from the fungi exhibited against human pathogens, it has also been found that several fungi species logged excellent inhibitory effects against plant pathogens in current use. This study was undertaken to screen the crude extracts of soil fungi, *Talaromyces flavus* Bodhi003, *Neosartorya fischeri* Bodhi 004, and *Eurotium* sp. Bodhi005 to evaluate the mycelial growth inhibition activity against ten plant pathogenic fungi which have caused severe diseases in economic crops in Thailand.

MATERIALS AND METHODS

Isolation and identification of soil fungi

Soil samples were collected from the riparian forest at Bodhivijjalaya College, Srinakharinwirot University, Sakaeo campus. Fungi were isolated, according to Boonsang *et al*. (2014). Briefly, one gram of soil was placed in a sterile test tube and incubated at 65°C for 15 minutes in water bath. The soil particles were transferred into sterile Petri dishes and poured with warm glucose ammonium nitrate agar contained with streptomycin sulfate. Then, they were incubated in the dark conditions at room temperature for 3 days. Hyphal tips of pure fungal were transferred onto Potato dextrose agar (PDA) slant and stored for further identification.

Preparation of the soil fungal extracts

Three species of soil fungi including *Talaromyces flavus* Bodhi003,*Neosartorya fischeri* Bodhi004, and *Eurotium* sp. Bodhi005 were extracted according to Dethoup *et al*. (2015), Erlenmeyer flasks (1L) containing 200 g of cooked rice were autoclaved. Selected soil fungi mycelial were inoculated into the flasks and incubated at 25°C for 30-days. After the fungi growth, the sample was macerated with ethyl acetate for 7-days at room temperature and then filtrated with Whatman No.1 filter paper to give the organic solutions and then evaporated under reduced pressure to furnish the crude ethyl acetate extracts of each soil fungus sample.

Plant pathogenic fungi isolate

Ten plant pathogenic fungi were isolated using tissue transplanting technique from several host plants including *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl., *Sclerotium rolfsii* (Sacc.) causing basal stem rot, *Phytophthora palmivora* (E.J. Butler) causing root rot in durian, *Colletotrichum capsici* (E.J. Butler and Bisby) causing anthracnose in chili, *Pyricularia grisea* Sacc. causing rice blast, *Alternaria* sp. causing fruit rot, *Helminthosporium maydis* (Y. Nisik and C. Miyake) causing southern corn leaf blight, *Rhizoctonia solani* (J.G Kuhn) causing sheath blight, *Fusarium oxysporum* (E.F.Sm. and Swingle) causing fusarium wilt and *Colletotrichum gloeosporioides* (Penz. and Sacc.) causing anthracnose. All fungi stock cultures were maintained on PDA slant and stored at -20°C.

In vitro antifungal activity test

Dilution plate method was used for the evaluation of the *in vitro* antimycelial growth of plant pathogenic fungi. The concentrations of 1, 10, 100, 1,000 and 10,000 ppm of each extract were tested according to Boonsang *et al*. (2014). One mL of each extract solutions was added into 9 mL of warm PDA, then mixed and poured into the Petri dishes. The plates were inoculated with mycelium of the plant pathogenic fungi and incubated at 25°C for 7-14- days. Each treatment was performed with five replications in a complete randomized design. The effectiveness of the antifungal activity was assessed based on the percentage of mycelial growth inhibition

which was calculated using the formula: G1-G2 / G1 × 100, where G1 is the colony radius of the fungi in control, and G2 the colony radius of plant pathogenic fungi in treatments.

Data analyses

Data obtained were submitted to the analysis of variance (ANOVA) according to Jantasorn *et al*. (2016) and means were compared by Duncan's multiple range test ($P<0.05$) using the statistical program SPSS version 16 (IBM Corporation, Somers, NY).

RESULTS AND DISCUSSION

The data indicates reduction in growth of ten plant pathogenic fungi in response to the tested *T. flavus* Bodhi003, *N. fischeri*Bodhi004, and *Eurotium* sp. Bodhi005 extracts. The growth of each of the ten plant pathogenic fungi was found to have an inverse relationship with the concentrations of three extractions seven days after inoculation. The antifungal activity of *T. flavus* Bodhi003 crude extract revealed completed inhibition of the growth of *L. theobromae, S. rolfsii* and *P. grisea* at a concentration of 10,000 ppm when compared with the control (Table.1). However, the same concentration was more efficient in the growth inhibition of *Alternaria* sp. and *R. solani* on it at 61.11% and 82.22% respectively. Additionally, the antifungal activity of *T. flavus* Bodhi003 crude extract was observed as 50% mycelial growth inhibition against *S. rolfsii* at a concentration of 1,000 ppm.

N. fischeri Bodhi004 crude extract also recorded antifungal activity, 100% growth inhibition against *P. palmivora, P. grisea, Alternaria* sp. and *R. solani* at a concentration of 10,000 ppm and was more efficient in reducing the mycelial growth of *S. rolfsii, C. capsici* and *F. oxysporum* (Table.2). However, the extract was effective at a concentration of 1,000 ppm against two plant pathogenic fungi, displaying a strong activity against *P. palmivara* and *R. solani*. The plate treated with low concentrations of crude extracts, failed to inhibit the growth of mycelia.

Finally, at concentrations of 10,000 ppm of *Eurotium* sp. Bodhi005 crude extract showed 100% mycelial growth inhibition against *L. theobromae, S. rolfsii, P. palmivora, P. grisea* and *C. gloeosporioides* and it remained highly effective against three species of plant pathogenic fungi with more than 50% inhibition, namely, *Alternaria* sp., *R. solani* and *F. oxysporum* (Table. 3). Despite, at a concentration of 1,000 ppm, *Eurotium* sp. Bodhi005 showed antifungal activity when tested, thus causing a complete inhibition of mycelial growth in *S. rolfsii*. However, two crude extracts, namely *N. fischeri*Bodhi004 and *Eurotium* sp. Bodhi005, were the most effective in reducing the mycelial growth of eight plant pathogenic fungi when compared with *T. flavus* Bodhi003 crude extract.

Table 1. *In vitro* inhibitory effect of *Talaromyces flavus* Bodhi003 crude extract at various concentrations on the mycelial growth of ten plant pathogenic fungi.

Pathogens	Mycelial growth inhibition at concentrations (ppm) (%)				
	1	**10**	**100**	**1,000**	**10,000**
Lasiodiplodia theobromae	0^{j}	0^{j}	0^{j}	13.33^{h}	100^{a}
Sclerotium rolfsii	0^{j}	0^{j}	0^{j}	50^{d}	100^{a}
Phytophthora palmivora	0^{j}	0^{j}	0^{j}	20^{g}	48^{d}
Colletotrichum capsici	0^{j}	0^{j}	0^{j}	0^{j}	24^{f}
Pyricularia grisea	0^{j}	0^{j}	5.56^{i}	16.67^{h}	100^{a}
Alternaria sp.	0^{j}	0^{j}	0^{j}	22.67^{fg}	61.11^{c}
Helminthosporium maydis	0^{j}	0^{j}	0^{j}	15.11^{h}	14.67^{h}
Rhizoctonia solani	0^{j}	0^{j}	0^{j}	15.56^{h}	82.22^{b}
Fusarium oxysporum	0^{j}	0^{j}	0^{j}	0^{j}	37.78^{e}
Colletotrichum gloeosporioides	0^{j}	0^{j}	0^{j}	0^{j}	6.67^{i}

Means followed by the same letter do not significantly different at $P<0.05$, when analysed using Duncan's multiple range test of One-Way ANOVA.

Table 2. *In vitro* inhibitory effect of *Neosartorya fischeri* Bodhi004 crude extract at various concentrations on the mycelial growth of ten plant pathogenic fungi.

Pathogens	%mycelial growth inhibition at concentrations (ppm)				
	1	10	100	1,000	10,000
Lasiodiplodia theobromae	0^{o}	0^{o}	0^{o}	0^{o}	0^{o}
Sclerotium rolfsii	0^{o}	0^{o}	0^{o}	17.78^{k}	88.89^{b}
Phytophthora palmivora	0^{o}	0^{o}	0^{o}	60.44^{c}	100^{a}
Colletotrichum capsici	0^{o}	0^{o}	0^{o}	5.56^{n}	54.44^{e}
Pyricularia grisea	0^{o}	0^{o}	8.89^{m}	24.22^{i}	100^{a}
Alternaria sp.	5.56^{n}	5.56^{n}	12.78^{l}	30^{g}	100^{a}
Helminthosporium maydis	0^{o}	0^{o}	0^{o}	24.44^{i}	23.33^{ij}
Rhizoctonia solani	0^{o}	0^{o}	0^{o}	52.67^{f}	100^{a}
Fusarium oxysporum	0^{o}	0^{o}	10^{m}	27.56^{h}	57.78^{d}
Colletotrichum gloeosporioides	0^{o}	0^{o}	0^{o}	0^{o}	10^{m}

Means followed by the same letter do not significantly different at $P<0.05$, when analysed using Duncan's multiple range test of One-Way ANOVA.

Table 3. *In vitro* inhibitory effect of *Eurotium sp.* Bodhi003 crude extract at various concentrations on the mycelial growth of ten plant pathogenic fungi.

Pathogens	%mycelial growth inhibition at concentrations (ppm)				
	1	10	100	1,000	10,000
Lasiodiplodia theobromae	0^{t}	0^{t}	0^{t}	0^{t}	100^{a}
Sclerotium rolfsii	12.22^{n}	18.89^{h}	25.56^{g}	100^{a}	100^{a}
Phytophthora palmivora	0^{t}	0^{t}	5.56^{r}	37.78^{e}	100^{a}
Colletotrichum capsici	0^{t}	0^{t}	0^{t}	0^{t}	18.22^{i}
Pyricularia grisea	10^{o}	4.89^{s}	6.22^{q}	13.11^{m}	100^{a}
Alternaria sp.	0^{t}	0^{t}	8.89^{p}	16.67^{k}	57.22^{d}
Helminthosporium maydis	0^{t}	0^{t}	8.89^{p}	17.56^{j}	27.78^{f}
Rhizoctonia solani	0^{t}	0^{t}	0^{t}	15.56^{l}	82.22^{b}
Fusarium oxysporum	0^{t}	0^{t}	0^{t}	0^{t}	73.33^{c}
Colletotrichum gloeosporioides	0^{t}	0^{t}	0^{t}	6.67^{q}	100^{a}

Means followed by the same letter do not significantly different at $P<0.05$, when analysed using Duncan's multiple range test of One-Way ANOVA.

The most effective crude extract is found to be *Eurotium* sp. Bodhi005 which recorded growth inhibitions against seven plant pathogenic fungi namely, *L. theobromae, S. rolfsii, P. palmivora, P. grisea, R. solani, F. oxysporum* and *C. gloeosporioides* when compared with the water control. Interestingly, the crude extracts of *Eurotium* sp. Bodhi005 exhibited excellent inhibitory activity against two plant pathogenic fungi same as plant extracts at a concentration of 10,000 ppm (Jantasorn *et al.,* 2016). In another investigation, the antifungal activity from plant extracts was tested on different plant pathogenic fungi and has been reported by various researchers (Abd-Ellatif *et al.,* 2011; Ghasemi *et al.,* 2012; Bahraminejad *et al.,* 2013; Salehan *et al.,* 2013).

The highest antifungal activity against *P. palmivora, P. grisea, Alternaria* sp. and *R. solani* was reported with *N. fischeri* Bodhi004 crude extract at a concentration of 10,000 ppm. The efficacies of the *Neosartorya* species in inhibiting the mycelial growth of plant pathogenic fungi have been reported earlier. Boonsang *et al.* (2014) also reported that the *N. pseudofischeri* KUFA0060 and *N. quadricincta* KUFA0064 crude extracts inhibited the mycelial growth of *P. palmivora* and *C. capsici* at a concentration of 100 ppm. While the crude extracts of *N. fischeri* Bodhi004, *T. flavus* Bodhi003 and *Eurotium* sp. Bodhi005 inhibited mycelial growth of

plant pathogenic fungi with higher antifungal activity than any of the other plant extracts (Suriani *et al.,* 2015; Olufolagi *et al.,* 2015). On the other hand, the extracts of *T. flavus* Bodhi003 and *Eurotium* sp. Bodhi005 were found to inhibit the mycelial growth of *L. theobromae* and *S. rolfsii* even at the concentration of 10,000 ppm. Our results reveal that the extract of soil fungi exhibited antifungal potential as determined by the mycelial growth inhibition of these plant pathogenic fungi, especially the extracts of *Eurotium* sp. Bodhi005 since it showed a significant inhibition of the mycelial growth and was recorded at 100 % against *S. rolfsii* at a concentration of 1,000 ppm. In addition, the antifungal activity of the crude extract against *S. rolfsii* revealed a promising antifungal activity for the extract of *Eurotium* sp. Bodhi005 when compared to previous reports. Aimn *et al.* (2013) also reported that the efficacies of tobacco leaf and turmeric rhizome extracts against *S. rolfsii*, but these two extracts were considerably less active than the crude extract of *Eurotium* sp. Bodhi005 which displayed a complete inhibition of the mycelial growth at 1,000 ppm. The soil fungi extracts are a bio-pesticide control agent that is safe for usage with respect to people and environmental accumulation. The extractions are able to control the plant pathogens and prevent the pathogens from developing resistance to fungicide (Derbalah *et al.,* 2011).

The antifungal activity of the *T. flavus* Bodhi003, *N. fischeri*Bodhi004, and *Eurotium* sp. Bodhi005 extracts in this study indicate that the extractions exhibit the highest potential for mycelial growth inhibition on plant pathogenic fungi that cause diseases on economic crops in Thailand. The crude extracts obtained from *N. fischeri* Bodhi004 and *Eurotium* sp. Bodhi005 could provide promising sources in the development antifungal agents against eight plant pathogenic fungi*viz., L. theobromae, S. rolfsii, P. palmivora, P. grisea, Alternaria sp., R. solani, F. oxysporum* and *C. gloeosporioides*. For the biological management of plant diseases, the potential of soil fungi extracts could be used in the prevention and control of the growth of plant pathogenic fungi and may be applied as alternative methods to reduce the use of chemical fungicides.

ACKNOWLEDGEMENTS

This work was financially supported by the Strategic Wisdom and Research Institute, Srinakharinwirot University, under project number 754/2558. We also thank Assistant Professor Dr. Tida Dethoup, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University for technical support.

REFERENCES

Abdel-Fattah, G.M., El-Haddad, S.A., Hafez, E.E. and Rashad, Y.M. 2011. Induction of defense responses in common bean plants by *Arbuscular mycorrhizal* fungi. *Microbiological Research*, **166**(4): 268–281

Abd-Ellatif, S., Abdel Rahman, S. M. and Deraz, S. F. 2011.Promising antifungal effect of some folkloric medicinal plants collected from El- Hammam habitat, Egypt against dangerous pathogenic and toxinogenic fungi. *Journal of Agricultural and Biological Science*, **6(9)**: 25–32.

Agrios, G.M. 2005. Plant Pathology. 5th ed. AP, New York, NY, 922 **PP**.

Aimn, R., Sarker, B.C., Adhikary, S.K., Sultana, S. and Zubair, T. 2013. Effect of some botanical extracts and cow's urine on *Sclerotiumrol fsii* causal agent of foot and root rot of betel vine. *The International Journal of Engineering and Science*, **2(9)**:77-82.

Al-Askar, A. A. 2012. In Vitro antifungal activity of three Saudi plant extracts against some phytopathogenic fungi. *Journal of Plant Protection Research*, **52**(**4)**: 458-462.

Alwathnani, H.A. and Perveen, K. 2012. Biological control of fusarium wilt of tomato by antagonist fungi and cyanobacteria. *African Journal of Biotechnology*, **11(5)**: 1100–1105.

Bahraminejad, S., Amiri, R., Ghasemi, S. and Fathi, N. 2013. Inhibitory effect of some Iranian plant species against three plant pathogenic fungi. *International Journal of Agricultural Crop Sciences*, **5(9)**: 1002-1008.

Boonsang, N., Dethoup,T., Singburaudom, N., Gomes, N.G.M. and Kijjoa, A. 2014. *In vitro* antifungal activity screening of crude extracts of soil fungi against plant pathogenic fungi. *Journal of Biopesticides*, **7**: 156-166.

Couch, B.E. and Kohn, L.E. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, **94**: 683-693.

Derbalah, A. S., El-Mahrouk, M. S. and El-Sayed, A. B. 2011. Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*. *Plant Pathology Journal*, **10(3)**: 115-121.

Dethoup, T., Kumla, D. and Kijjoa, A. 2015. Mycocidal activity of crude extracts of marine-derived beneficial fungi against plant pathogenic fungi. *Journal of Biopesticides*, **8(2)**:107-115.

Eamvijarn, A., Kijjoa, A., Bruyere, C., Mathieu, V., Manoch, L., Lefranc, F., Silva, A., Kiss, R. and Herz, W. 2012.Secondary metabolites from a culture of the fungus *Neosartoryapseudo fischeri* and their *in vitro* cytostatic activity in human cancer cells. *Planta Medica*, **78(16)**:1767-1776.

Eamvijarn, A., Gomes, N.M., Dethoup, T., Buaruang, J., Manoch, L., Silva, A., Pedro, M., Marini, I., Roussis, V. and Kijjoa, A. 2013. Bioactive meroditerpenes and indole alkaloids from the soil fungus *Neosartorya fischeri* (KUFC 6344), and the marine-derived fungi *Neosartoryalaciniosa* (KUFC 7896) and *Neosartoryatsunodae* (KUFC 9213). *Tetrahedron*, **69(70)**: 8583-8591.

Ghasemi, S., Abbasi, S., Bahraminejad, S. and Harighi, B. 2012. Inhibitory effect of some plant crude extracts against cucumber damping-off agents. *Australasian PlantPathology*, **41(3)**: 331-338.

Jantasorn, A., Moungsrimuangdee, B. and Dethoup, T. 2016. *In vitro* antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases. *Journal of Biopesticides*, **9(1)**:1-7

Kaewchai, S., Soytong, K. and Hyde, K.D. 2009.Mycofungicides and fungalbio-fertilizers. *Fungal Diversity*, **38**: 25-50.

Olufolaji, D.B., Adeosun, B.O. and Onasanya, R.O. 2015. *In vitro* investigation on antifungal activity of some plant extracts against *Pyricularia oryzae*. *Nigerian Journal of Biotechnology*, **29**:38-43

Salehan, N. M., Meon, S. and Ismail, I.S. 2013. Antifungal activity of *Cosmos caudatus* extracts against seven economically important plant pathogens. *International Journal of Agriculture and Biology*, **15(5)**: 864-870.

Sibounnavong, P., Sibounnavong, P., Kanokmedhakul, S. and Soytong, K. 2012.Antifungal activities of *Chaetomiumbrasilense* CB01 and *Chaetomiumcupreum* CC03 against *Fusarium oxysporum* f.sp. *lycopersici*race 2. *Journal of Agricultural Technology*, **8(3)**:1029-1038.

Suriani, N. L., Suprapta, D. N., Sudana, I. M. and Temaja, R. M. 2015. Antifungal activity of *Piper caninum* against *Pyricularia oryzae* Cav. The cause of rice blast disease on rice. *Journal of Biology, Agriculture and Healthcare*, **5(8)**:72-78.

Talubnak, C. and Soytong, K. 2010. Biological control of vanilla anthracnose using *Emericellanidulans. Journal of Agricultural Technology*, **6(1)**: 47-55.

Tapwal, A., Nisha, Garg, S., Guatam, N. and Kumar, R. 2011. *In Vitro* antifungal potency of plant extracts against five phytopathogens. *Brazilian Archives Biology and Technology*, **54(6)**: 1093-1098.

[1*]Jantasorn, A., Mongon, J., Moungsrimuangdee, B. and Oiuphisittraiwat, T.

[1]Bodhivijjalaya College, Srinakharinwirot University, 114 Sukhumvit 23, Bangkok, Thailand, 10110; [*]Corresponding author: aromj@g.swu.ac.th